BRIEFER ARTICLES

HETEROTHALLISM IN BREAD MOLD, *RHIZOPUS NIGRICANS*

In recent publications the writer has shown that, according to their method of sexual reproduction, the Mucorineae may be divided into two groups—homothallic and heterothallic: In a homothallic species, zygospores are formed from branches of the same mycelium and can be obtained from the sowing of a single spore. In heterothallic species, which probably constitute the large majority of the Mucorineae, zygospores are formed from branches which necessarily come from two different mycelia belonging to sexually opposite races or strains. By sowing a single spore, therefore, one cannot obtain zygospores of a member of the heterothallic group unless the culture becomes contaminated with spores of the opposite strain.

Within the last few months, two communications have appeared which claim that the production of the zygospores of Rhizopus is not at all dependent upon the presence of two sexual races; in other words, that on the proper nutrient the zygospores can be obtained from the sowing of a single spore. This laboratory weed, the common bread mold, is the best known species among the mucors and has been used as a type of the heterothallic group.

A propos of the article by HAMAKER (Science N. S. **23**:710. 1906), the writer has recently summarized the results of a series of investigations on the sexual condition in this species covering a period of over six years (Science N. S. **24**:118–122. 1906). Since this was written, the preliminary communication of HAMAKER has been reviewed in the BOTANICAL GAZETTE (**42**:77. 1906) where the detailed formula is repeated for making the corn muffin bread claimed to be a sure medium for the production of the zygospores of this species. It is not the purpose of the writer to repeat what has been said already in regard to the inadequacy of this nutrient theory of zygospore formation. It seems desirable, however, to consider somewhat in detail the paper of NAMYSLOWSKI (*Rhizopus nigricans* et les conditions de la formation de ses zygospores: Bull. Acad. Sci. Cracovie 676–692. *pl. 21.* 1906), since this investigator explicitly casts doubt upon the occurrence of heterothallism in any of the Mucorineae and would give the appearance of supporting his conclusions by cultural experiments with Rhizopus.

It may be said at the start that, as every laboratory mycologist knows, Rhizopus shares with Penicillium the distinction of being the worst fungus weed in laboratory cultures, and is practically certain to come as a contamination of substrata rich in carbohydrates unless they be sterilized and *kept* under sterile conditions. In the Harvard laboratory, as in most other botanical institutions, Rhizopus is obtained for class use by spontaneous infection of bread. Moreover, the sporangium wall is brittle and when ruptured the spores are discharged into the air. It is somewhat difficult, therefore, to obtain a pure transfer from a single sporangium without contamination with spores of other sporangia near by, and, before the discovery of heterothallism in this form, the writer more than once obtained zygospores from transfers which at the time were thought to have come from single sporangia.

NAMYSLOWSKI'S conclusions are based for the most part on two series of cultures. In the first, 19 bread cultures were made from spores from one single sporangium, and zygospores were produced in every case. In the second series, 46 single spores were isolated by the separation method and used in the inoculation of cultures chiefly of bread. Of these 6 were destroyed by bacteria, 13 produced only a feeble growth of mycelium (probably also infected by bacteria), 13 only sporangia, and 14 zygospores. The failure to obtain zygospores in so large a number of the sowings is ascribed to improper moisture content of the air in the cultures and to other unfavorable conditions which are not further investigated.

Two explanations are possible for these results: (1) that NAMYSLOWSKI was in fact dealing with a homothallic form of Rhizopus and zygospores were the result of single spore sowings; (2) that infection from the air brought the (+) and (−) strains into contact and caused the production of zygospores. Mr. H. A. EDSON, of the Vermont Experiment Station, has recently sent the writer zygospores of Rhizopus which he writes were secured synthetically by opposing two strains, one obtained from Vermont, the other from Virginia. A dozen strains were tested by him from as many different sources, but failed to produce zygospores alone in pure cultures. The writer himself has tested 60 different strains from localities in such widely separated parts of the world as North and South America, England, Germany, France, Honolulu, and the Philippine Islands, and has found none which alone in pure cultures will produce zygospores. A homothallic form of the ordinary species, therefore, would seem *a priori* rather improbable, and to be proven only by careful cultures strictly under sterile conditions.

NAMYSLOWSKI says nothing about sterilizing his culture media, and the

fact that out of 46 cultures on bread, 6 (over 13 per cent.) were destroyed by bacteria and 13 more were probably also infected by bacteria, since they produced only a scanty mycelium devoid of fructifications, shows that NAMYSLOWSKI'S cultures were far from pure, and renders it probable that, in the 14 instances in which zygospores were obtained out of the 46 cultures, the (+) and (−) strains had become present through infection; while in the 13 cultures in which only sporangia were obtained, whatever infection occurred was of the same strain as the spores used in the inoculation. The 19 cultures on bread from the same sporangium would give zygospores if the inoculating material became infected by spores of the opposite race before sowing, even though subsequent infection did not occur.

That zygospores may be obtained by accidental infection of a favorable substratum with both (+) and (−) spores has been repeatedly demonstrated by the writer's own experience in the Harvard laboratory. Professor CAMPBELL, moreover, has written that for ten years he has gotten zygospores by spontaneous infection of suitable substrata, and an investigation showed that in zygosporic material sent from his laboratory only the two (+) and (−) strains were present which alone were incapable of zygospore formation.

Since the above was written, Dr. NAMYSLOWSKI has kindly sent the writer zygosporic material of the *Rhizopus nigricans* which he used in his experiments. Inoculations were at once made into sterilized nutrient and a large number of the young zygospores resulting were isolated and laid on cleared nutrient agar in Petri dishes. Paired cultures X & XX and Y & YY were obtained by making mycelial transfers from the outgrowths from the suspensors of two zygospores both of whose suspensors had germinated. On the assumption that the species is heterothallic, each pair should contain the two opposite strains, and each strain when grown alone should be incapable of zygospore formation. In addition, cultures Z & ZZ were obtained from suspensors that could not be traced with certainty to the same zygospore, and cultures A–D were obtained from the germination of single isolated sporangiospores. By opposing inoculations from these cultures against one another and against standard (+) and (−) test strains, it was seen that X and Y are (−) and XX, YY, Z, ZZ, and A–D are (+). The contrasts numbered over 40 and were made for the most part on sterilized flour paste in stender dishes, but were controlled by cultures on nutrient agar in test tubes. None of the strains produced zygospores on substrata favorable to zygospore formation when sown alone in pure cultures, nor when sown together with the same strain from a different source. They have always formed them, however, whenever the opposite strains are grown

together under proper nutrient conditions. The form is therefore heterothallic.

Whether or not it may ever be found that a homothallic race may occur in a species normally heterothallic (perhaps not an impossible condition in view of the writer's having obtained a homothallic mycelium of the heterothallic Phycomyces), the evidence at hand leads one to the conclusion that the large majority of the Mucorineae are heterothallic.—A. F. BLAKESLEE, *Cryptogamic Laboratory, Harvard University.*

TWO EMBRYO-SAC MOTHER CELLS IN *LILIUM LONGIFLORUM*

(WITH ONE FIGURE)

The presence of more than one archesporial cell in the megasporangium of the Spermatophyta has frequently been reported for gymnosperms and dicotyledons, but the records of their occurrence in monocotyledons are very few. In 1882 GUIGNARD[1] figured two small hypodermal cells in *Ornithogalum pyrenaicum*, which he interprets as archesporial. As these cells are but little larger than the other cells of the nucellus, and the character of the protoplasmic content of the adjacent cells is not shown, the evidence here is not altogether conclusive. COULTER and CHAMBERLAIN[2] state that they have observed two preparations of *Lilium philadelphicum* showing respectively three and five archesporial cells, but they neither described nor figured them. BERNARD[3] observed and figured two four-celled embryo sacs

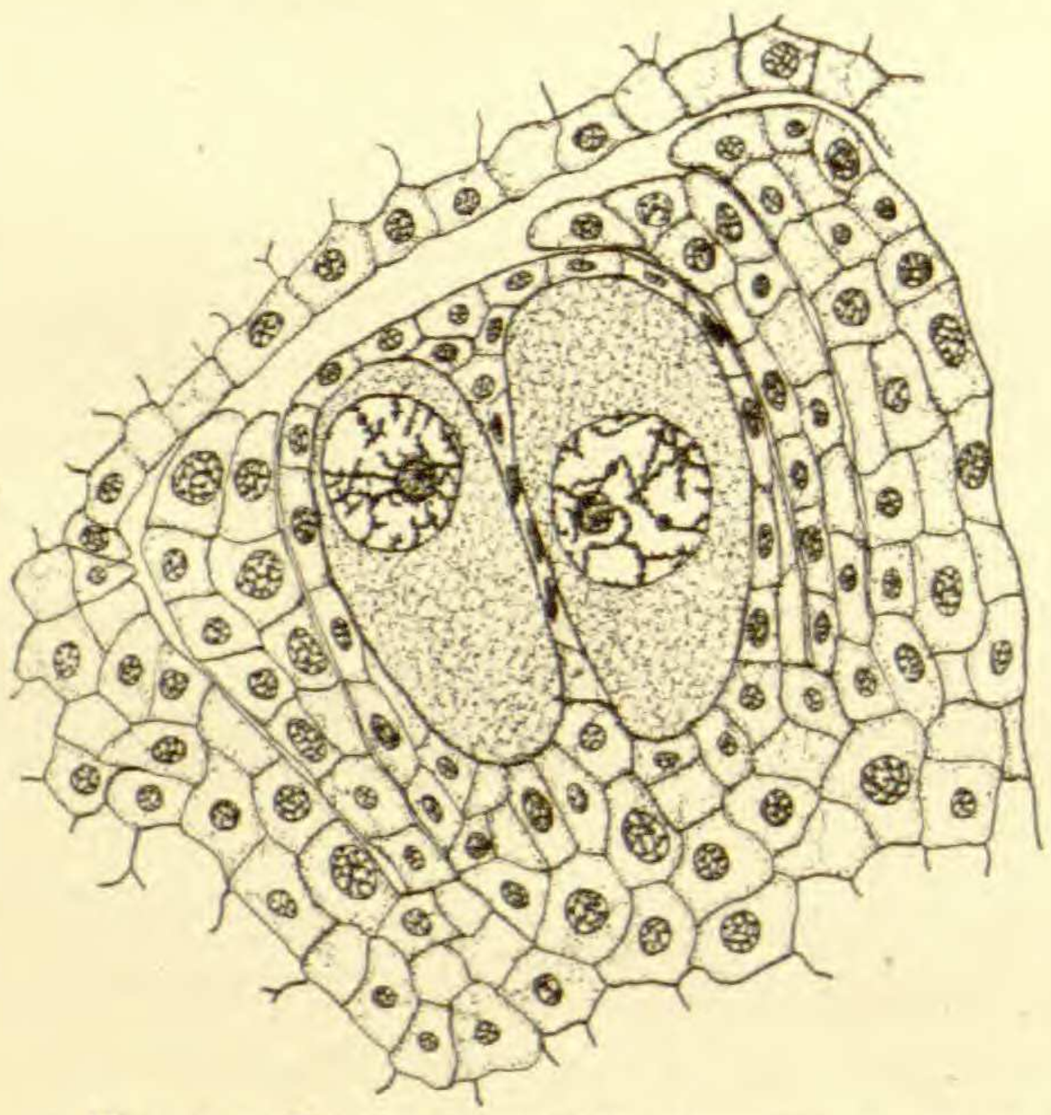

FIG. 1.—Vertical section through a young ovule of *Lilium longiflorum*, showing two embryo-sac mother cells.

[1] GUIGNARD, L., Recherches sur le sac embryonnaire des Phanérogames Angiospermes. Ann. Sci. Nat. Bot. VI. **13**:136–199. *pls. 3–7.* 1882.

[2] COULTER and CHAMBERLAIN, Morphology of Angiosperms. New York. 1903.

[3] BERNARD, C. H., Recherches sur les sphères attractives chez *Lilium candidum*, etc. Jour. Botanique **14**:178. *pls. 4–5.* 1900.